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Cover: Whale Shark *Rhincodon typus* and Reef - made with poster colours. © P. Kritika.

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SHORT COMMUNICATION

Incidence of *Clinostomum complanatum* (Trematoda: Clinostomidae) in *Trichogaster fasciata* (Actinopterygii: Osphronemidae), the first report from Deepor Beel, Assam, India

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Abstract: Fish constitute a major component of diet for the people of northeastern India and one of the main food items for most people in Assam. But fishes are facing serious risks due to parasitic infestations which deteriorate the food value and also leads to fish mortality. The present study aims to investigate the occurrence of helminth parasites in some selected fishes of the species *Mystus tengara*, *Channa punctata*, and *Trichogaster fasciata* collected from Deepor Beel, Guwahati, Assam. Trematode parasite species have been recorded from *Trichogaster fasciata*. The recovered trematode is *Clinostomum complanatum* belonging to the family Clinostomatidae. The present study incorporates the morphological, morphometric, and scanning electron microscopy studies of the prevailing parasite. This study is the first report on the occurrence of helminth parasites in the edible freshwater fishes collected from Deepor Beel.

Keywords: Food value, freshwater fish, Helminth, Infestation, parasite, Trematode.

India occupies its position among the 17 mega-diversity countries harboring around 55 families of freshwater fish (Koiri & Roy 2016). In northeastern India, Assam is the most water-resourceful state that has a rich variety of ichthyofauna and the beels (lakes) produce 50,000 tons of fish per year (Dutta & Lahon 1987; Lebanan & Mohilal 2021). Fish constitute a major component of diet of the people of northeastern India (Jyrwa et al. 2016) and one of the main food items for most people in Assam. Moreover, fish and fish cultivation provide a valuable source of food and employment opportunities. Also, fish constitutes the most beneficial and nutritional resource for human beings (Wali et al. 2016). But fishes are being threatened by various parasitic infestations throughout the world. Parasitic infestations cause deterioration of the food value of fish and may even lead to fish mortality (Keisham & Mohilal 2020; Moravec et al. 2017). Parasitic diseases of fish are prevalent and are of specific importance in the tropics. About 30,000 helminth species have been estimated as parasites of fishes, of which most are known to be of serious threat to their hosts. As estimated by the World Health Organisation (WHO), the number of people currently infected with fish-borne trematodes exceeds 18 million, and many more are at risk (Jyrwa et al. 2016). They pose serious problem causing huge economic losses in the fishing industry and in aquaculture (Amare et al. 2014). Helminth parasites constitute a significant group of pathogens and cause infection & diseases in

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fish in freshwater & marine environments (Jyrwa et al. 2016). Considerable work has been conducted on several aspects of fish faunal diversity, hydrobiology, and productivity of rivers and beels of Guwahati of Assam (Dey 1981; Das & Bordoloi 1997; Saha & Bordoloi 2009). Bhalerao (1942) has made a remarkable contribution to the taxonomy of digenetic trematode parasites.

In the context of Assam, a few works have been contributed to fish parasitology. Binky et al. (2011) studied the helminth parasites of Karbhala Wetland in Cachar District of Assam. Das et al. (2012) studied the intensity of cestode parasites in *Monopterus cuchia* of Cachar. Das & Goswami (2014) studied the organal distribution and seasonal occurrence of parasites from three wetlands of Goalpara District. Ngasepam & Kar (2014) worked on the helminth parasites of fishes of Sone beel in Karimganj. Singha et al. (2015) studied the parasites of *Notopterus notopterus, Channa punctata,* and *Heteropneustes fossilis* in Dolu Lake, Silchar. The present study was undertaken to study the presence of any helminth parasites in some selected edible freshwater fishes from Deepor beel, Guwahati, Assam that were not reported hitherto from this area.

Materials and Methods

During the present study, fishes of the species *Mystus tengara, Channa punctata*, and *Trichogaster fasciata* were collected from September 2020 to August 2021 from Deepor Beel with the help from local fishermen. During the study about 200 fishes were collected and examined. Fish samples were brought to the laboratory in live condition. Serial numbers were provided for each sample, the total length and weight measured of the specimens thoroughly examined for the parasitological study. In the present study, the body cavity, kidneys, liver, stomach, and intestines were examined for the presence of endoparasites. Trematodes were collected from the body cavity of *Trichogaster fasciata* by following Justine et al. (2012). The recovered parasites were flattened between two slides, fixed in 70% ethanol, and processed for wholemount preparation following standard procedure using Aceto alum carmine as a stain. *Clinostomum complanatum* were identified by light microscopy following Keys to Trematoda (Vol. 2) and the morphological description of *C. complanatum* by Caffara et al. (2011) and Ngamniyom et al. (2012). The morphometric measurements of the parasite were taken by using a stage and ocular micrometer.

Preparation of the specimen for Scanning Electron Microscopy (SEM)

For Scanning Electron Microscopy (SEM), the parasite specimens were fixed in 2.5% glutaraldehyde, washed in 0.1M Sodium Cacodylate Buffer for 4 hours at 4˚C before post-fixing them in 1% Osmium Tetroxide in the same buffer for 1 hour at 4˚C. The specimens were then dehydrated through a graded series of acetone, dried, and mounted on brass stubs. After mounting, the specimens were coated with gold and examined with a FESEM scanning electron microscope model Zeiss Sigma 300.

Results and Discussion

A total of eighty-five middle-size *Trichogaster fasciata* were examined during the study period, and 27 were found infested with the specimen of *Clinostomum complanatum* (Image 1a,b,c). All specimen were recovered from the peritoneal cavity of the examined fishes.

Description of trematode parasite, *Clinostomum complanatum*

Identifying characters: The fixed worms appear relatively stout; however, prior to fixation, the live

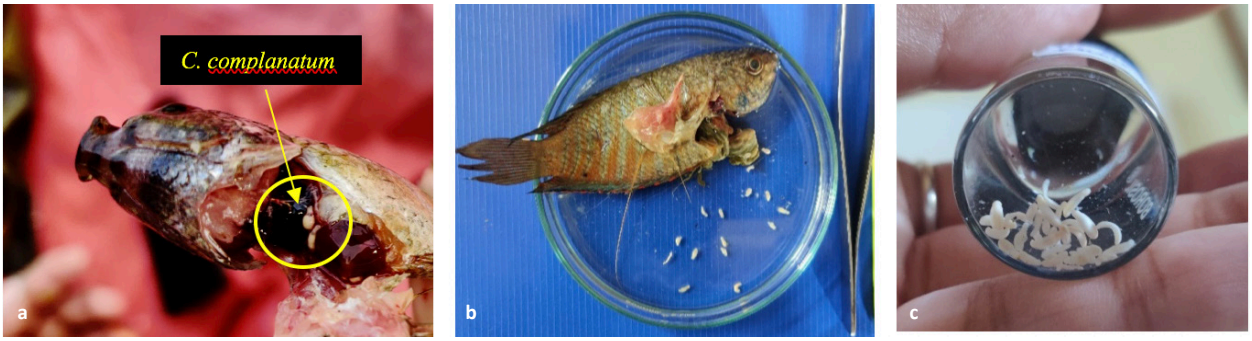

Image 1. *Clinostomum complanatum*: a—infected *T. fasciata* | b—collected in a petri dish | c—metacercariae stored in 70% ethanol. © Bobita Bordoloi.

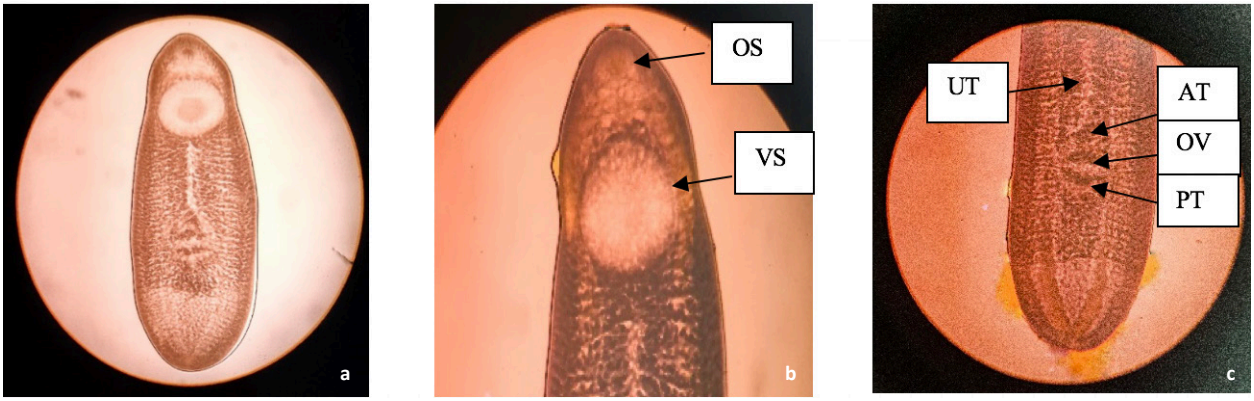

Image 2. Light microscopic structure of *Clinostomum complanatum*: a—Whole structure | b—Structure showing the position of anterior sucker (AS) and ventral sucker (VS) | c—Structure showing the position of the anterior testis (AT), posterior testis (PT), uterus (UT), and ovary (OV). © Bobita Bordoloi.

worms are very motile, exhibiting high contractility. The worm-like bodies are medium-sized to large, stout, linguiform, tongue-shaped, and bluntly rounded at the anterior and posterior ends. The body length varies between 5.7–7 mm, and the body width 1.9–2.5 mm. The body is convex dorsally and concave ventrally. Oral sucker oval, subterminal, and surrounded by collar-like folds and its surface is covered by ridges and pits. Oral sucker length ranges between 0.098–0.175 mm while the width varies between 0.200–0.395 mm. Ventral sucker muscular, well-developed, and larger than the oral positioned in the anterior half of the body. The length of the ventral sucker ranges between 0.700–0.900 mm, and the width ranges between 0.810–0.930 mm. Caeca long, simple, located in the anterior half of the body without long lateral branches and diverticula. Testes, located in posterior half of the body are smooth or irregular in shape. The anterior lobe of the testes lies in the middle, and the posterior lobe is positioned at the rear end of the parasite. The length of the anterior testis varies between 0.307–0.520 mm, and the width ranges between 0.290–0.480 mm. Posterior testis length varies between 0.292–0.500 mm, and the width varies between 0.450–520 mm. Ovary intertesticular, ovoid or rounded, median or submedian. The length of the ovary ranges between 0.149–0.164 mm, and the width ranges between 0.119–0.168 mm. Vitelline follicles are present between the posterior extremity and the level of the ventral sucker. The uterus is intercaecal, positioned between the caudal region of the ventral sucker and intertesticular space.

Scanning electron microscopy structure: Scanning electron microscopy of the trematode parasite revealed additional topographical features that confirmed the specimen as *Clinostomum complanatum*. The body surface is characterized by the presence of tegumental pits and furrows with a smooth, aspinous layer. The body has rounded extremities with two suckers (one oral sucker and one ventral sucker) (Image 3a,b). Oral sucker subterminal in position with a rounded opening characterized by two collar rings and covered by ridges, pits, and dome-like papillae (Image 3c). The rim of both the oral and the sucker were aspinous and non-papillated. The oval-shaped ventral sucker positioned in the anterior half of the body exhibited sponge-like characters, and wavy wrinkles with dome-shaped papillae around the ventral sucker (Image 3d,e). The tegumental infoldings, furrows, and ridges impart stretching and contractility to the metacercariae. The dorsal surface of the parasite body revealed regularly distributed spinous protrusions (Image 3f).

The family Clinostomidae was first erected by Lühe (1901) for *Clinostomum* Leidy, 1856. In the present study, the morphometric measurements of the parasite corroborate with the study of Caffara et al. (2011) and Ngamniyom et al. (2012). Scanning electron microscopic studies of the topography of the parasite revealed additional features which correlates with the study by Abidi et al. (1988), Caffara et al. (2011), Ngamniyom et al. (2012), & Kundu et al. (2021) and confirmed the recovered parasite specimen as *Clinostomum complanatum* belonging to the family Clinostomatidae. However, Ngamniyom et al. (2012) and Caffara et al. (2011) observed spines with cytoplasmic ridges in the dorsal and the ventral region showing cobblestone-like units, but such observations were not recorded in the trematode parasites in the present study.

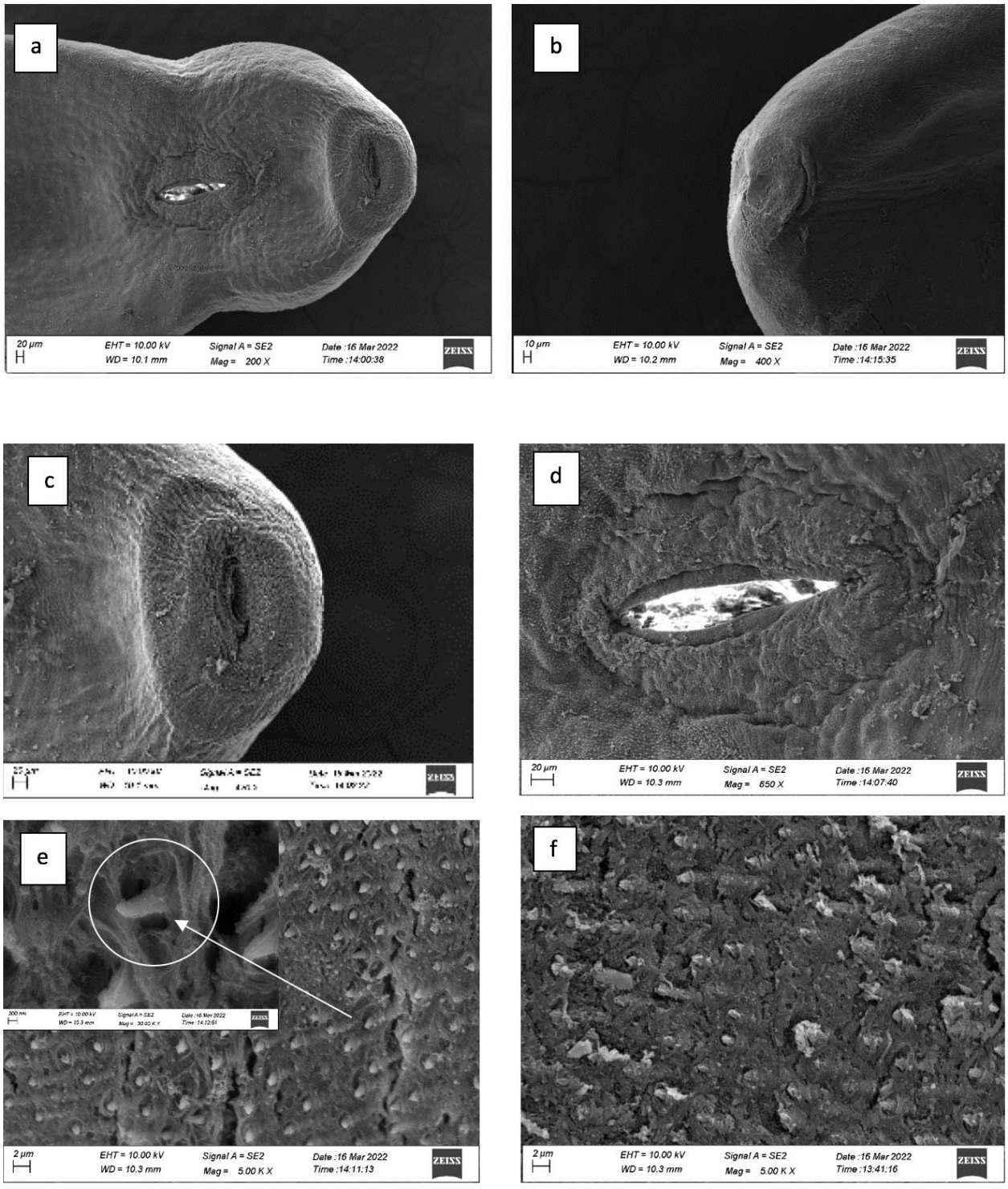

Image 3. Scanning electron microscopic structure of *Clinostomum complanatum*: a—Metacercariae showing the presence of anterior sucker (AS) and ventral sucker (VS) | b—Rounded posterior region of the metacercariae | c—Subterminal oral sucker surrounded by two collar rings and covered by ridges and pits | d—Ventral sucker surrounded by sponge-like characters, wavy wrinkles and spinous structures | e—Ventral surface of the metacercariae showing spinous characters | f—Dorsal surface of the metacercariae showing somewhat spinous structures.

Conclusion

The present study is the first report on the occurrence of trematode parasite *Clinostomum complanatum* from edible freshwater fish species *Trichogaster fasciata* collected from Deepor Beel. The edible freshwater fish *Trichogaster fasciata* were infected with the metacercariae of the trematode parasite. Along with morphological data, SEM study provides specific characteristics of the topography that helped in the identification of the trematode parasite.

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